Seasonal photosynthesis and anthocyanin production in 10 broadleaf evergreen species

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Abstract. Leaves of many evergreen species turn red when exposed to high sunlight during winter due to production of photoprotective anthocyanin pigments, while leaves of other species, lacking anthocyanin, remain green. Why some evergreen species synthesise anthocyanin pigments while others do not is currently unknown. Furthermore, the relative photosynthetic performance of anthocyanic (red) and acyanic (green) evergreens has yet to be described. Here we present seasonal ecophysiological data for five red and green broadleaf evergreen species. We hypothesise that species which synthesise anthocyanins in winter leaves correspond to those with the most drastic seasonal photosynthetic declines, as reduced energy sinks increase vulnerability to photoinhibition and need for photoprotection. Our results did not support this hypothesis, as gas exchange measurements showed no difference in mean seasonal photosynthetic capacity between red- and green-leafed species. Consistent with anthocyanin's shading effect, red-leafed species had significantly higher chlorophyll content, lower chlorophyll *a*/*b* ratios, and higher maximum light capture efficiency of PSII (F_v/F_m) than green-leafed species during the winter, but not during the summer (when all leaves were green). We conclude that anthocyanin production during winter is likely not associated with diminished photosynthetic capacity, and may simply represent an alternative photoprotective strategy utilised by some species during winter.

Additional keywords: chlorophyll, photoinhibition, photoprotection, pigments, winter.

Introduction

Evergreen plants exhibit a broad range of adaptations enabling extended photosynthetic carbon gain during winter months, despite a variety of abiotic stresses (for reviews see Tranquillini 1964; Nilsen 1992; Uemura and Steponkus 1999; Bigras and Colombo 2001; Öquist and Hüner 2003; Adams *et al.* 2004). Of particular importance are adaptations which allow photosynthetic tissues to avoid and/or dissipate excess light energy (Krause 1994). Because low temperatures inhibit the biochemical reactions of the Calvin cycle, but do not significantly affect light capture, high-sunlight exposure in combination with low temperatures may lead to excessive energy captured relative to that used in carbon fixation (Hüner *et al.* 1998). This imbalance leads to an increase in energy and electron transfer to molecular oxygen, production of radical oxygen species, and increased vulnerability to photo-oxidative damage (Mittler 2002). Adaptations which curtail these processes generally do so by either reducing photosynthetically active radiation (PAR) incident on the leaf or chloroplasts (i.e. avoidance strategies) or quenching/dissipating absorbed energy before transfer to the chlorophyll reaction centres (non-photochemical quenching). Anthocyanin pigments are an example of an avoidance strategy, as they are thought to intercept incident blue-green light and dissipate the absorbed quantum energy as heat, thereby protecting underlying photosynthetic cells from photoinhibition (Lee and Gould

2002). This shade function has been supported by studies showing reduced photosynthesis in red-leafed morphotypes of several species relative to green (e.g. Bahler *et al.* 1991; Burger and Edwards 1996; Gould *et al.* 2002) as well as reduced photoinhibition (e.g. Feild *et al.* 2001; Hughes *et al.* 2005; Liakopoulos *et al.* 2006), often resulting in elevated photosynthesis in anthocyanic leaves relative to acyanic leaves under photoinhibitory conditions (e.g. Gould *et al.* 1995; Liakopoulos *et al.* 2006).

Although the capability for synthesising anthocyanins is nearly ubiquitous among angiosperms, not all evergreen angiosperms synthesise anthocyanins in leaves during winter when photoprotective mechanisms are generally highly engaged (Adams *et al.* 2004). Interestingly, many of the evergreen species which do not synthesise anthocyanin in winter leaves do so in other tissues or during different ontogenetic stages, such as in juvenile leaves, flowers, stems, roots, senescing leaves, and/or in response to pathogen infection (Table 1). Their failure to produce anthocyanins in winter leaves therefore suggests that anthocyanins are not necessary or beneficial for these species during the winter season. However, this assumption has not been tested, and no explanation currently exists for why some broadleaf evergreen species synthesise anthocyanins in winter leaves while others do not.

One possibility is that anthocyanin synthesis only occurs in species with drastic seasonal decreases in photosynthetic

Table 1. Descriptions of the 10 broadleaf evergreen species used in the study

Species	Family	Plant form	Origins	Anthocyanic tissues
Galax urceolata (Poir.) Brummitt[A]	Diapensiaceae	Forb/herb	Native	Winter rhizomes and petioles, winter, juvenile and injured leaves
Leucothoe fontanesiana (Steud.) Sleumer[A]	Ericaceae	Shrub	Native	Winter stems, winter, juvenile, and injured leaves
Lonicera japonica (Thunb.)[A]	Caprifoliaceae	Vine	Introduced	Runners, winter leaves
Hexastylis shuttleworthii (Britten & Baker)[A]	Aristolochiaceae	Forb/herb	Native	Winter leaves, winter petioles, flowers
Rhododendron spp. (horticultural azalea)[A]	Ericaceae	Shrub	Introduced	Flowers, new growth stems, senescing leaves
Kalmia latifolia (L.)	Ericaceae	Shrub	Native	Juvenile and injured leaves, stems, flowers
Rhododendron maximum (L.)	Ericaceae	Shrub	Native	Glands on juvenile leaves and stems, developing stems, flowers
Vinca minor (L.)	Apocynaceae	Vine/groundcover	Introduced	Flowers
Rhododendron catawbiense (Michx.)	Ericaceae	Shrub	Native	Flowers, senescing leaves
Rhododendron spp. (horticultural azalea)	Ericaceae	Shrub	Introduced	Flowers, new growth stems, senescing leaves

[A]Synthesises anthocyanin in winter leaves.

capacity during the winter, corresponding with an increased need for photoprotection. A decreased capacity for photosynthesis is known to render plants intrinsically more vulnerable to photoinhibition, due to a reduction in energy sinks available for energy dissipation (i.e. photochemical quenching; Osmond 1981; Powles 1984). This idea has been supported by previous research on anthocyanins in deciduous tree species, where it has been shown that shade-intolerant species (characterised by high maximum photosynthesis) tend to not produce anthocyanins during the autumn, while shade-adapted species (lower maximum photosynthesis) do (Koike 1990; Hoch *et al*. 2001). Indeed, evergreen species are known to vary greatly in seasonal photosynthetic capacity due to either differences in seasonal growth, and/or general differences in intrinsic limitations to photosynthesis (e.g. vulnerability to cavitation, water-stress tolerance, freezing damage) (Davis *et al*. 1999; Uemura and Steponkus 1999; Verhoeven *et al*. 1999; Adams *et al*. 2002; Taneda and Tateno 2005). However, it is unknown whether such differences correspond with red/green leaf coloration in winter.

To determine whether colour change corresponds with any observable patterns in seasonal photosynthesis, we measured photosynthetic gas exchange, photosynthetic light response curves, and maximum light capture efficiency of PSII (F_v/F_m) for 10 broadleaf evergreen species (representing five different plant families) differing in their production of anthocyanin during winter.

Materials and methods

Sites and species

Species selected for study were mature field plants growing along sun-exposed roadsides in Jonas Ridge, NC, USA (35°57′20″N, 81°53′55″W; altitude: ~1200 m) on south or south-east-facing sites receiving >6 h full sunlight (i.e. >1350 mol m^{-2} s^{-1} on a horizontal surface) per day during both summer and winter months. Measurements were taken on clear sunny days, with little or no cloud cover. Detailed descriptions of the individual species examined are in Table 1. All hourly time designations are expressed as Solar Time (List 1971).

Photosynthetic gas exchange

Winter photosynthesis measurements were taken between 4 December 2005 and 4 March 2006, and 15–17 December 2007, and summer measurements 2–4 August 2007. All measurements were made on first-year leaves under full ambient sunlight (>1350 mol m^{-2} s^{-1}), and plants were sampled via a standard random walk procedure (Codling and Hill 2005). Measurements were replicated at times of the day that corresponded to periods with the highest stomatal conductance. During the summer, these higher conductance periods occurred in early morning (between 0800 and 1100 hours) and late afternoon (1600 and 1900 hours) when air temperatures were coolest. During the winter, measurements were taken in mid-afternoon (1100 until 1500 hours), when air temperatures were the warmest. A portable photosynthesis system (PP. Systems Inc., model TPS-1, Amesbury, MA, USA) was used to measure leaf conductance, photosynthetic CO_2 assimilation, leaf temperatures, and air temperature for gas exchange measurements. This instrument was chosen because its small size facilitated the substantial climbing involved in sampling. TPS gas exchange values were compared with those derived using a LI-COR model Li-6400 (Li-Cor, Inc., Lincoln, NE, USA), and were found to be within ±3%.

Photosynthetic response to light was measured in the field on warm winter days (daytime maximum air temperatures >17°C) following at least three consecutive days with night time temperatures >2°C. Measurements were made at the same times of day described above for instantaneous measurements using a Li-Cor 6400 infrared gas analyser with red and blue LED light sources (Li-Cor, Inc.). This device was used for light-response curves because the LED transmittance curve avoids the absorbance spectrum of anthocyanin, allowing photosynthesis

to be measured without the pigment's shading effect (LED spectrum illustrated in fig. 8-3 of Li-6400 Instruction Manual); this allowed light exposure to be standardised for red and green leaves. The temperature of the leaf cuvette was set to 20°C during the winter to normalise measurements between samples, while summer measurements were taken at ambient temperatures (29–35°C). Measurements were initiated at ambient photosynthetic photon flux densities (PPFD), then increased up to 2000 mol m^{-2} s^{-1}, and then decreased incrementally until irradiance was zero. All measurements were taken on plants that had been exposed to full sunlight for at least 2 h to ensure maximal stomatal opening (checked by measuring leaf conductance values). The order of individual plant measurements was randomised each day and sample size was at least $n = 3$, with different individuals tested for each species each day.

Chlorophyll fluorescence

Maximum light capture efficiency of PSII (F_v/F_m) was measured in the field on mature, current-year leaves between 4 December 2005 and 4 March 2006, and 15 December 2006 and 15 March 2007, and in summer 2–4 August 2007. Measurements were randomised according to plant and leaf, and taken between 1100 and 1400 hours, representing the time of day when photoinhibition of photosynthesis was highest. A PAM Fluorescence System (Hansatech Institute, model FMS-2, Cambridge, UK) emitting a two second long, 3 mmol m^{-2} s^{-1}, 594 nm saturating pulse was used to derive F_v/F_m for all sampled leaves. Prior to each measurement, plants were dark-adapted for 30 min using model FMS-2 leaf clips.

Pigment analyses

Chlorophyll concentrations were measured and *a/b* ratios were calculated to assess the relative emphasis of light capture *v.* processing in winter leaves. Increased chlorophyll content is associated with an emphasis on light capture, as well as increased chlorophyll *a/b* ratios, which reflect an increase in core (exclusively chl *a*) relative to light-harvesting (both chl *a* and *b*) complexes, and/or higher ratios of PSI (4/1 ratio of chl *a/b*) to PSII (1.2/1 ratio) (Cui *et al.* 1991; Demmig-Adams 1998; Hopkins and Hüner 2004).

On 16 February 2007, one mature (fully-expanded) and healthy-appearing leaf was removed from five separate individuals in the field between 1100 and 1300 hours, after all plants had been exposed to full sunlight for at least 3 h. A standard hole puncher was used to excise three 0.33 cm^2 leaf discs, which were immediately placed in 3 mL *N,N'*-dimethylformamide to extract in the dark for 24 h. Chlorophyll concentrations were determined spectrophotometrically using the equations described by Porra (2002). From the same leaves, three additional leaf discs were excised and placed in 3 mL 6 M HCl : H$_2$O : MeOH (7 : 23 : 70) for 24 h at 4°C to extract anthocyanins. Anthocyanin concentration was measured spectrophotometrically as $A_{530} - 0.24$ A_{653} using an extinction coefficient of 30 0001 mol^{-1} cm^{-1} (Murray and Hackett 1991). All extracts were analysed using a Hewlett Packard 8453 UV-VIS spectrophotometer (Hewlett Packard, Palo Alto, CA, USA).

Statistics

Linear regression analysis was used to evaluate correlations between temperature and photosynthesis, leaf conductance, and F_v/F_m, for each species. Slopes and Y-intercepts of best-fit lines for these values were grouped according to the presence or absence of anthocyanin (i.e. red or green winter leaf colour, respectively) and compared using a two-sample, two-tailed Student's *t*-test (Zar 1999). The effects of leaf colour on F_v/F_m, photosynthesis and leaf conductance values were analysed using a nested MANOVA (with species nested within colour), on three winter dates differing in air temperature (*Cold*: low temp −9°C, high –3°C; *Mild*: low −3°C, high 10°C; and *Warm*: low 8°C, high 20°C; dates are given in the legend of Fig. 2); measurements were also made on one summer day for comparison (low 31°C, high 18°C). Measurements on different days used random leaves sampled from multiple (i.e. >5) plants throughout the plot, invalidating use of the repeated-measures technique (Zar 1999).

For light saturation curves, nested MANOVAs were also used to compare dark respiration, light-saturated photosynthesis at ambient CO$_2$ (A$_{sat}$), and photosynthesis (A) at individual irradiance levels for species differing in colour. A one-tailed *t*-test was used to compare linear slopes of photosynthesis below light saturation for red and green species during summer and winter. To compare seasonal photosynthetic capacity, summer A$_{sat}$ (i.e. maximum values observed during the summer) were compared with A$_{sat}$ observed on a warm winter day (low 8°C, high 20°C, representing the maximum values observed during the winter) for each species using a one-tailed *t*-test. A one-tailed Student's *t*-test was also used to compare mean seasonal percent declines in A$_{sat}$ for red and green species. Photopigment concentrations and chlorophyll *a/b* ratios of red and green species were compared using a two-way nested MANOVA, with species nested within leaf colour. All tests employed met distributional and sample size requirements described in Zar (1999), and significance was accepted at $P < 0.05$.

Results

Winter photosynthesis

Photosynthesis significantly ($P < 0.0001$) increased with air temperature for all species during the winter (r^2 values for green species: *Kalmia latifolia* (L.) 0.583, *Rhododendron maximum* (L.) 0.475, *Rhododendron catawbiense* (Michx.) 0.711, *Vinca minor* (L.) 0.627, *Rhododendron* azalea spp. 0.766; r^2 values for red species: *Galax urceolata* (Poir.) Brummitt 0.428, *Leucothoe fontanesiana* (Steud.) Sleumer 0.627, *Lonicera japonica* (Thunb.) 0.772, *Hexastylis shuttleworthii* (Britten & Baker) 0.779, *Rhododendron* azalea spp. 0.857) (Figs 1 and 2). The slope of this increase ($m = 0.219$) did not statistically differ between red and green species as a group ($P = 0.827$).

Leaf conductance was more variable during the winter than photosynthesis, but increased significantly with temperature for all species except the green-leafed *R. maximum* and *V. minor* during the winter ($P = 0.99$ and 0.383 for *R. maximum* and *V. minor*, respectively, $P < 0.05$ for all remaining species; Fig. 1). The slope of the increase in conductance according to
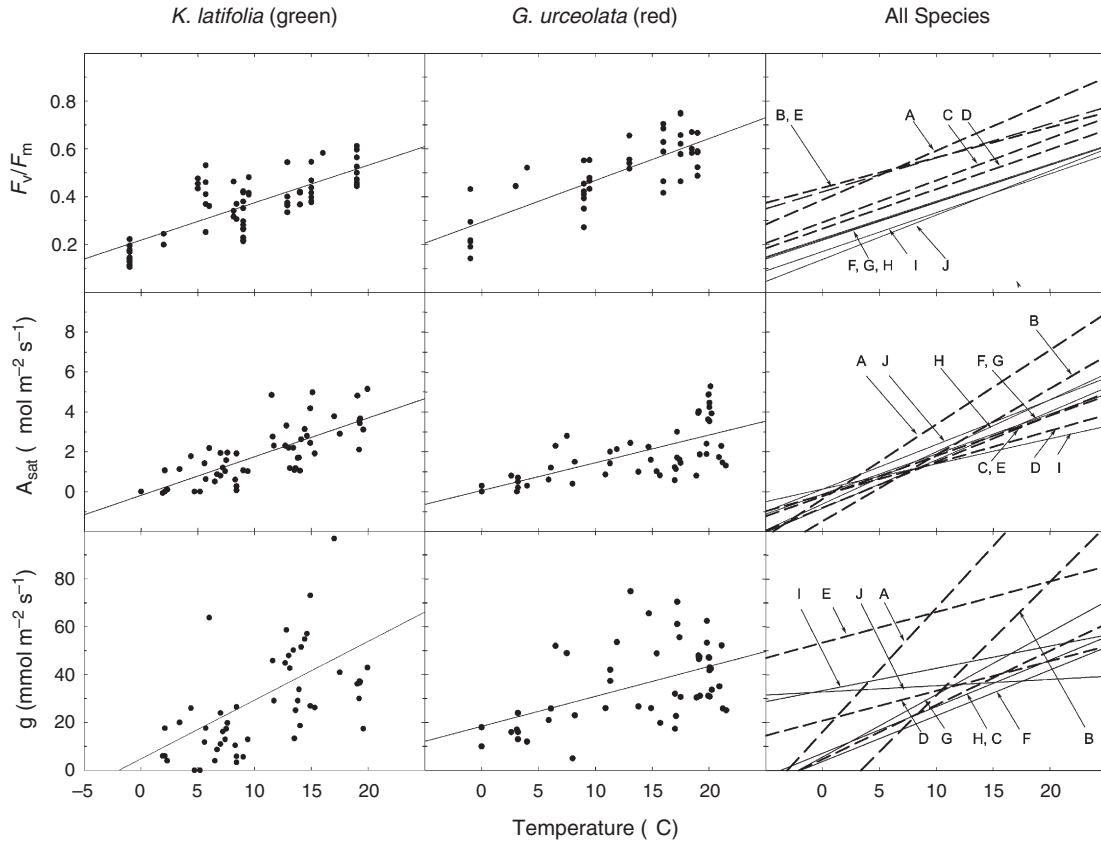
Fig. 1. Winter maximum light capture efficiency of PSII (F_v/F_m), light-saturated photosynthesis (A_{sat}), and leaf conductance (g) for red (dashed lines) and green (solid lines) broadleaf evergreen species measured across a range of ambient temperatures. Left column: representative green species (*Kalmia latifolia*), middle column: representative red species (*Galax urceolata*), right column: best fit lines of all red (dashed) and green (solid) species measured. Red species: *Lonicera japonica* (A), *Rhododendron* azalea spp. (B), *Galax urceolata* (C), *Leucothoe fontanesiana* (D), *Hexastylis shuttleworthii* (E). Green species: *Rhododendron catawbiense* (F), *Kalmia latifolia* (G), *Rhododendron* azalea spp. (H), *Rhododendron maximum* (I), *Vinca minor* (J).

temperature during the winter ($m = 1.87$) did not significantly differ for red and green species ($P = 0.136$).

On cold winter sample days, photosynthesis for all plants was at or near zero, corresponding with near-zero leaf conductance values (Fig. 2). There was no significant difference in A_{sat} or leaf conductance between species differing in leaf colour on a cold winter day ($P = 0.303$ and 0.754, respectively) or a mild winter day ($P = 0.101$ and 0.607). However, on warm days, red-leafed species photosynthesised at significantly higher rates than green-leafed species ($P < 0.0001$), and also had significantly higher leaf conductance ($P < 0.0001$). However, there was also a significant species effect on warm days ($P < 0.0001$), with very high A_{sat} and g values for the red-leafed *L. japonica*, and very low values for the green-leafed *V. minor* driving statistical differences between groups; values for other species generally overlapped (Fig. 2).

For light-response curves (Fig. 3), red species had significantly higher A_{sat} than green species during the winter ($P = 0.01$), although again, there were highly significant interspecific differences ($P < 0.0001$), and with the exception of *L. japonica* and red *Rhododendron* azalea spp., A_{sat} for red- and green-leafed species was comparable. Similarly, there was

no difference in A_{sat} between red- and green-leafed species during the summer when all leaves were green ($P = 0.75$). During winter, red-leafed species also showed significantly higher A than green-leafed species at low PAR values (i.e. <500 mol m^{-2} s^{-1}) ($P < 0.01$ at 200 and 100 mol m^{-2} s^{-1}), and had significantly greater quantum yield of photosynthesis at low PAR (as evidenced by steeper slopes below the light saturation point) ($P = 0.02$, where mean m for red species $= 0.016$, and green species $= 0.0098$); the groups did not differ in any of these parameters during the summer ($P > 0.50$). During both summer and winter seasons, dark respiration rates of red- and green-leafed species did not differ ($P > 0.30$).

Seasonal differences in photosynthetic gas exchange

While winter photosynthesis of all species was significantly less than summer values on both cold and mild days ($P < 0.0001$ for both), on the warmer days (air temperature $>16°C$) some species were able to photosynthesise at rates that did not significantly differ from summer rates (green species: *V. minor* $P = 0.09$; red species: *G. urceolata* $P = 0.52$; Fig. 4). The remaining species photosynthesised significantly below summer levels on
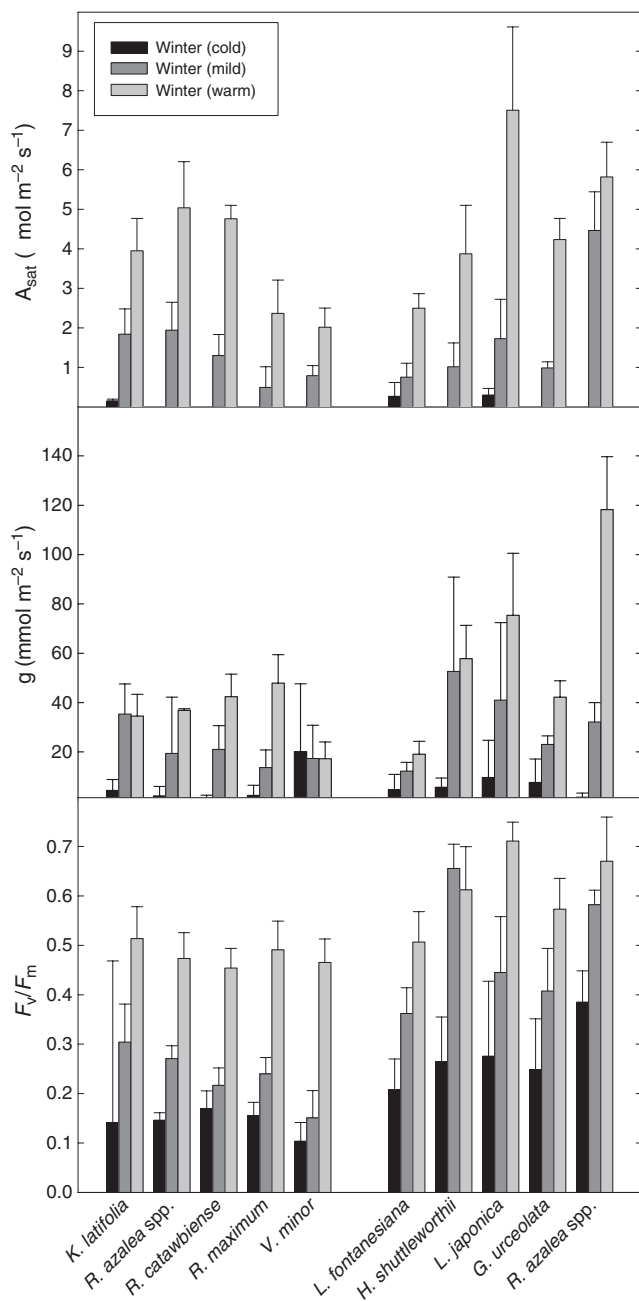
Fig. 2. Cold, mild, and warm winter day measurements of A_{sat}, g, and F_v/F_m for green-leafed and red-leafed species. Bars represent means of 5–15 replicates ± s.d. Cold winter day measurements were taken on 29 January 2007 (low −9°C, high temp −3°C); mild, 7 February 2006 (low −3°C, high 10°C); warm, 15 December 2006 (low 8°C, high 20°C); summer measurements taken on 8 June 2006 (low 12°C, high 23°C).

warm winter days ($P < 0.01$). As a group, the mean percent seasonal decline in photosynthesis relative to summer values exhibited by red leaves (30%) did not significantly differ from green leaves (41%) ($P = 0.53$). At all temperatures measured, all 10 species exhibited significantly reduced leaf conductance of water vapour during the winter relative to the summer ($P < 0.0001$).

Fluorescence

Winter F_v/F_m values increased significantly ($P < 0.0001$) with temperature for all species (Fig. 1; r^2 values >0.56 for all green species; r^2 values >0.41 for all red species). F_v/F_m increased with temperature along the same slope ($m = 0.0158$) for both red and green groups ($P = 0.733$). F_v/F_m values of red species were consistently higher than those of green species across the temperature range measured, and Y-intercepts of regressions were significantly higher for red species as a group compared with green species ($P = 0.016$). On cold, mild, and warm winter days, red-leafed species had consistently higher (~0.15 units on average) mean F_v/F_m than green-leafed species ($P < 0.0001$ for all; Figs 1 and 2).

Pigments

Species with red winter leaves had significantly higher total chlorophyll (24% on average), and anthocyanin content than green leaves during the winter ($P < 0.0001$ for both; Table 2). On average, red species also had significantly (27%) lower chlorophyll a/b ratios than green species ($P < 0.0001$; Table 2).

Discussion

The hypothesis that species with red winter leaves would be characterised by lower photosynthetic capacity during winter than green-leafed species was not supported in this study. Mean maximum photosynthesis of red-leafed species during winter did not differ from green-leafed species on cold, mild, or relatively warm winter days (Figs 1 and 2). In fact, the two species with the highest photosynthesis values during winter were both red-leafed species (*L. japonica* and the red *Rhododendron* azalea spp.). Mean winter declines in photosynthesis relative to summer values (Fig. 4) also did not significantly differ between red-leafed species relative to green-leafed species, with some members of both groups having A_{sat} on warm winter days near values observed during the summer, and others exhibiting more drastic declines (i.e. <50% summer photosynthesis).

Fluorescence measurements of PSII were consistent with a photoprotective function of anthocyanin. Red-leafed species had consistently higher maximum light-capture efficiency of PSII (F_v/F_m) than green-leafed species across all measured temperatures during winter days (Figs 1 and 2); similar trends were also observed for pre-dawn measurements during the winter (data not shown). Because F_v/F_m is inversely correlated with sustained non-photochemical quenching (NPQ) and photoinhibition of photosynthesis, these results suggest that red-leafed species incurred less photo-oxidative damage, and thus engaged sustained NPQ to a lesser degree, than green-leafed species during the winter (Demmig-Adams *et al.* 1996). Although one may contend that red-leafed species could be utilising more the rapidly-reversible component of NPQ (which is lost during dark adaptation), we believe this to be unlikely given that red-leafed species also showed consistently higher quantum efficiency of PSII in the non-dark adapted state (φPSII) than green-leafed species during the winter (data not shown).

The shading effect of anthocyanin was also evidenced in the relatively shade-adapted light response curves of red compared
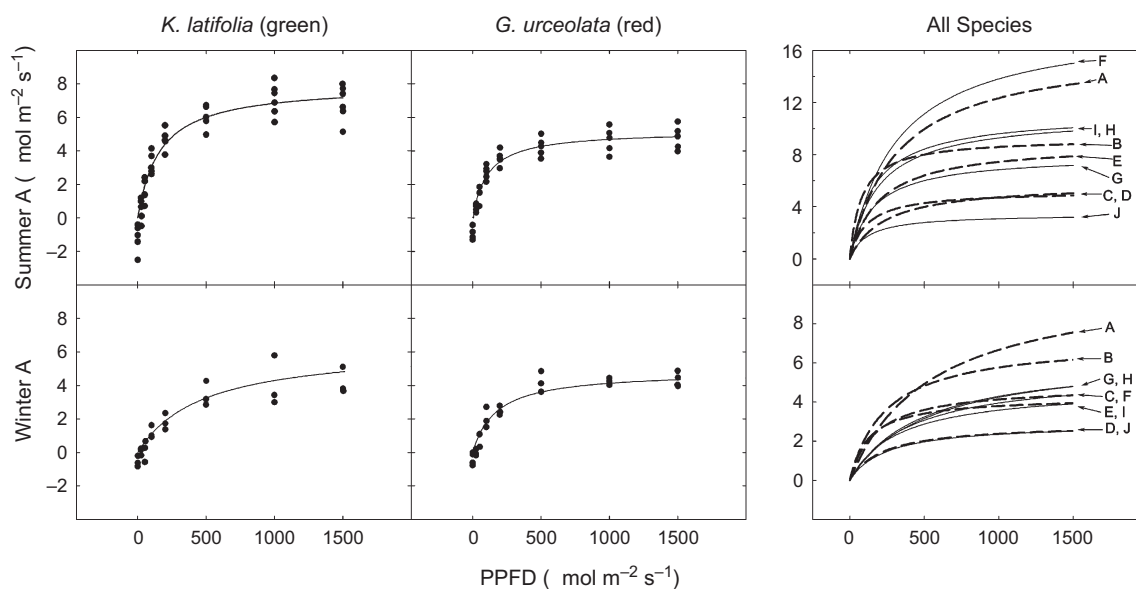
Fig. 3. Seasonal light response curves for red and green broadleaf evergreen species. Top row: summer; bottom row: winter. Left column: representative green species (*Kalmia latifolia*), middle column: representative red species (*Galax urceolata*), right column: best fit lines of all red (dashed) and green (solid) species measured. Red species: *Lonicera japonica* (*A*), *Rhododendron* azalea spp. (*B*), *G. urceolata* (*C*), *Leucothoe fontanesiana* (*D*), *Hexastylis shuttleworthii* (*E*). Green species: *Rhododendron catawbiense* (*F*), *K. latifolia* (*G*), *Rhododendron* azalea spp. (*H*), *Rhododendron maximum* (*I*), *Vinca minor* (*J*).
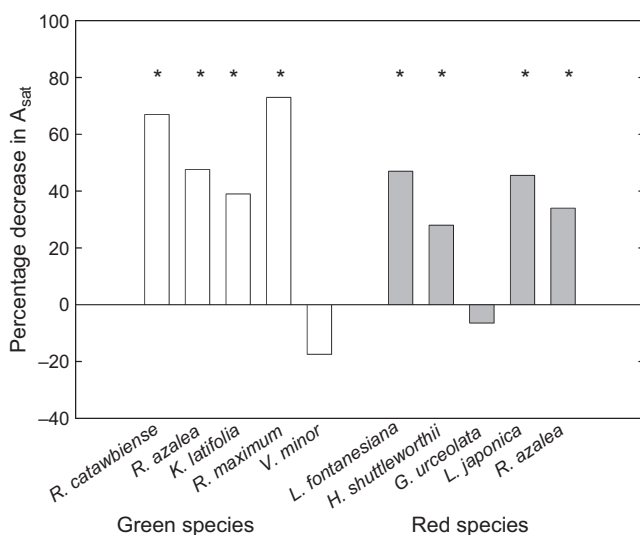

Fig. 4. Percent decrease in maximum observed winter A_{sat} relative to summer values. White bars represent green species, grey bars represent red species. Asterisks denote significant declines in maximum winter photosynthesis relative to summer values ($P < 0.05$).

Table 2. Winter photosynthetic and non-photosynthetic pigments
Content expressed per gram of fresh weight ($\pm$ SD). *Gaultheria procumbens* was used as a red representative instead of *Hexastylis shuttleworthii* due to limited availability of *H. shuttleworthii* leaves for destructive analyses

	Total chl (mg/g)	Chl *a/b*	Anthocyanin (mol/g)
Green spp. *Kalmia latifolia*	0.886 (0.22)	4.63 (0.88)	–
Rhododendron azalea spp.	0.810 (0.20)	4.45 (0.62)	–
Rhododendron catawbiense	0.987 (0.13)	4.29 (0.75)	–
Rhododendron maximum	0.793 (0.16)	3.61 (0.34)	–
Vinca minor	0.857 (0.14)	2.11 (0.30)	–
Red spp. *Leucothoe fontanesiana*	1.08 (0.29)	3.28 (0.34)	1.04 (0.32)
Lonicera japonica	0.623 (0.087)	3.10 (0.30)	2.61 (0.34)
Galax urceolata	1.61 (0.26)	2.21 (0.33)	3.27 (1.0)
Rhododendron azalea spp.	0.978 (0.19)	2.29 (0.28)	5.3 (1.4)
Gaultheria procumbens	1.10 (0.11)	3.07 (0.71)	1.05 (0.55)

with green leaves (Fig. 3). Under red and blue LED light (which does not overlap with the absorbance spectrum of anthocyanin), red-leafed species had significantly steeper slopes for the linear part of the light response curve (i.e. higher apparent quantum yield of photosynthesis) than green-leafed species and higher photosynthesis under low PAR (i.e. <500 mol m^{-2} s^{-1}) accordingly. The two groups did not differ in these respects during the summer, when all leaves

were green. Higher photosynthesis at low PAR is a common feature of more shade-adapted plants, reflecting either decreased respiration rates and/or a greater emphasis on light capture relative to light processing (Larcher 2003). Because dark respiration values were not significantly different between the red- and green-leafed groups during the winter, increased A_{sat} at low PAR was likely due to greater light capture capacity. Consistent with this explanation, total chlorophyll content was

significantly higher, and chlorophyll *a/b* ratios significantly lower in red- compared with green-leafed species (similar to results reported in Manetas *et al.* 2003; Lee *et al.* 2003; Hughes *et al.* 2005; Table 2).

Higher photosynthesis at lower light levels may also explain why red-leafed species were able to photosynthesise at saturated values under ambient sunlight, despite the shading effect of anthocyanin. Pietrini and Massacci (1998) estimated that anthocyanins at concentrations near the maximum values reported here (i.e. ~4.4 mol/g) may attenuate ~43% incoming PAR *in vivo*. Thus, under full sunlight, the amount of unabsorbed irradiance remaining should still be high enough to saturate photosynthesis (as all red-leafed species photosynthetically saturated near 25% full sunlight; Fig. 3). Perhaps anthocyanins reduce PAR transmittance sufficiently to curtail photoinhibition, but still transmit enough to allow the leaf to attain light saturation of photosynthesis, maximising sunlight capture and utilisation during the winter.

Because the red- and green-leafed species measured here did not differ in seasonal photosynthesis, we conclude that red coloration in winter leaves most likely does not correspond with diminished photosynthetic capacity. Instead, the comparable seasonal photosynthesis of red leaves compared with green suggests that photoprotection by anthocyanin represents an alternative (rather than additive) photoprotective strategy employed by some plants during the winter. Perhaps upregulation of anthocyanin compensates for species-specific deficiencies in other means of photoprotection (e.g. NPQ, antioxidants). Research is currently underway by the authors to investigate this possibility.

Alternatively, it is also possible that red-leafed species are indeed limited in photosynthetic capacity during the winter, but are able to photosynthesise at rates similar to green species due to anthocyanin's photoprotective effect. However, this would be difficult to determine without, for example, utilising anthocyanin-less mutants of red-leafed species.

Acknowledgements

References

Adams WW III, Demmig-Adams B, Rosenstiel TN, Brightwell AK, Ebbert V (2002) Photosynthesis and photoprotection in overwintering plants. *Plant Biology* **4**, 545–557. doi: 10.1055/s-2002-35434

Adams WW III, Zarter CR, Ebbert V, Demmig-Adams B (2004) Photoprotective strategies of overwintering evergreens. *Bioscience* **54**, 41–49. doi: 10.1641/0006-3568(2004)054[0041:PSOOE]2.0.CO;2

Bahler BD, Steffen KL, Orzolek MD (1991) Morphological and biochemical comparison of a purple-leafed and a green-leafed pepper cultivar. *HortScience* **26**, 736.

Bigras FJ, Colombo SJ (2001) 'Conifer cold hardiness. Tree physiology.' (Kluwer Academic Publishers: The Netherlands)

Burger J, Edwards GE (1996) Photosynthetic efficiency, and photodamage by UV and visible radiation, in red versus green leaf coleus varieties. *Plant & Cell Physiology* **37**, 395–399.

Codling EA, Hill NA (2005) Sampling rate effects on measurements of correlated and biased random walks. *Journal of Theoretical Biology* **233**, 573–588. doi: 10.1016/j.jtbi.2004.11.008

Cui M, Vogelmann TC, Smith WK (1991) Chlorophyll and light gradients in sun and shade leaves of *Spinacia oleracea. Plant, Cell & Environment* **14**, 493–500. doi: 10.1111/j.1365-3040.1991.tb01519.x

Davis SD, Sperry JS, Hacke UG (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. *American Journal of Botany* **86**, 1367–1372. doi: 10.2307/2656919

Demmig-Adams B, Adams WW III, Barker DH, Logan BA, Bowling DR, Verhoeven AS (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiologia Plantarum* **98**, 253–264. doi: 10.1034/j.1399-3054.1996.980206.x

Demmig-Adams B (1998) Survey of thermal energy dissipation and pigment composition in sun and shade leaves. *Plant & Cell Physiology* **39**, 474–482.

Feild TS, Lee DW, Holbrook NM (2001) Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. *Plant Physiology* **127**, 566–574. doi: 10.1104/pp.127.2.566

Gould KS, Kuhn DN, Lee DW, Oberbauer SF (1995) Why leaves are sometimes red. *Nature* **378**, 241–242. doi: 10.1038/378241b0

Gould KS, Vogelmann TC, Han T, Clearwater MJ (2002) Profiles of photosynthesis within red and green leaves of *Quintinia serrata. Physiologia Plantarum* **116**, 127–133. doi: 10.1034/j.1399-3054.2002.1160116.x

Hoch WA, Zeldin EL, McCown BH (2001) Physiological significance of anthocyanins during autumnal leaf senescence. *Tree Physiology* **21**, 1–8.

Hopkins WG, Hüner NPA (2004) Photosynthetic electron transport. In 'Introduction to plant physiology'. (Eds WG Hopkins, NP Hüner) pp. 68–71. (John Wiley: New York)

Hughes NM, Burkey KO, Neufeld HS (2005) Functional role of anthocyanins in high-light winter leaves of the evergreen herb, *Galax urceolata. The New Phytologist* **168**, 575–587. doi: 10.1111/j.1469-8137.2005.01546.x

Hüner NPA, Öquist G, Sarhan F (1998) Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224–230. doi: 10.1016/S1360-1385(98)01248-5

Koike T (1990) Autumn coloring, photosynthetic performance and leaf development of deciduous broad-leaved trees in relation to forest succession. *Tree Physiology* **7**, 21–32.

Krause GH (1994) Photoinhibition induced by low temperatures. In 'Photoinhibition of photosynthesis. From molecular mechanisms to the field'. (Eds NR Baker, JR Bowyer) pp. 331–348. (BIOS Scientific Publ.: Oxford)

Larcher W (2003) The light response of photosynthesis. In 'Physiological plant ecology'. pp. 111–120. (Springer: New York)

Lee DW, Gould KS (2002) Why leaves turn red. *American Scientist* **90**, 524–531. doi: 10.1511/2002.6.524

Lee DW, O'Keefe J, Holbrook NM, Feild TS (2003) Pigment dynamics and autumn leaf senescence in a New England deciduous forest, eastern USA. *Ecological Research* **18**, 677–694. doi: 10.1111/j.1440-1703.2003.00588.x

Liakopoulos G, Nikolopoulos D, Klouvatou A, Vekkos K-A, Manetas Y, Karabourniotis G (2006) The photoprotective role of epidermal anthocyanins and surface pubescence in young leaves of grapevine (*Vitis vinifera*). *Annals of Botany* **98**, 257–265. doi: 10.1093/aob/mcl097

List RJ (1971) 'Smithsonian Meteorological Tables.' (Smithsonian Institution Press: Washington D.C.) 527 pp.

Manetas Y, Petropoulou Y, Psaras GK, Drinia A (2003) Exposed red (anthocyanic) leaves of *Quercus coccifera* display shade characteristics. *Functional Plant Biology* **30**, 265–270. doi: 10.1071/FP02226

Mittler R (2002) Oxidative stress, antioxidants, and stress tolerance. *Trends in Plant Science* **7**, 405–410. doi: 10.1016/S1360-1385(02)02312-9

Murray JR, Hackett WP (1991) Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. *Plant Physiology* **97**, 343–351.

Nilsen ET (1992) Thermonastic leaf movements: a synthesis of research with *Rhododendron. Botanical Journal of the Linnean Society* **110**, 205–233.

Osmond CB (1981) Photorespiration and photoinhibition: some implications for the energetics of photosynthesis. *Biochimica et Biophysica Acta* **639**, 77–98.

Öquist G, Hüner NPA (2003) Photosynthesis of overwintering evergreen plants. *Annual Review of Plant Biology* **54**, 329–355. doi: 10.1146/annurev.arplant.54.072402.115741

Pietrini F, Massacci A (1998) Leaf anthocyanin content changes in *Zea mays* L. grown at low temperature: significance for the relationship between the quantum of PS II and the apparent quantum yield of CO_2 assimilation. *Photosynthesis Research* **58**, 213–219. doi: 10.1023/A:1006152610137

Porra RJ (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. *Photosynthesis Research* **73**, 149–156. doi: 10.1023/A:1020470224740

Powles SB (1984) Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology* **35**, 15–44. doi: 10.1146/annurev.pp.35.060184.000311

Taneda H, Tateno M (2005) Hydraulic conductivity, photosynthesis and leaf water balance in six evergreen woody species from fall to winter. *Tree Physiology* **25**, 299–306.

Tranquillini W (1964) The physiology of plants at high altitudes. *Annual Review of Plant Physiology* **15**, 345–362. doi: 10.1146/annurev.pp.15.060164.002021

Uemura M, Steponkus PL (1999) Cold acclimation in plants: relationship between the lipid composition and the cryostability of the plasma membrane. *Journal of Plant Research* **112**, 245–254. doi: 10.1007/PL00013882

Verhoeven AS, Adams WW III, Demmig-Adams B (1999) The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. *Oecologia* **118**, 277–287. doi: 10.1007/s004420050728

Zar JH (1999) 'Biostatistical analysis.' (Prentice Hall: Upper Saddle River, New Jersey, USA)